Molecular studies of HPE in Germany

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Here we report the first results of our HPE study to analyze genotype and phenotype of HPE patients in the German population. As a first step, the coding regions and the flanking intronic sequences of HPE3 (*SHH*), HPE2 (*SIX3*) and HPE5 (*ZIC2*) are amplified by PCR and screened for mutations by direct sequencing. Physicians are notified in reasonable time, so that the screening results can be applied for genetic counseling. If no mutation has been detected and the patients or there parents, respectively, are willing to participate, the patients samples and their clinical data are included in a collaborative research study.

The previously described *SHH* nonsense mutation Glu284(amber) (GAG->TAG) in Exon 3 was observed in an additional familial case presenting with microsigns of the HPE spectrum including global developmental delay, microcephaly, craniofacial dysmorphic features and solitary median maxillary central incisor (SMMCI). Both, the father as well as the oldest sister of the index patient also presented with SMMCI in combination with profound learning problems. In addition, bilateral choanal stenosis was observed in this sister. In another pregnancy of the mother a spontaneous abortion occurred in the 17. week of pregnancy, the fetus was reported to have an encephalocele. The observation of this *SHH* mutation further underlines the role of *SHH* in the pathogenesis of SMMCI as microsign of the HPE spectrum.

A new *SIX3* missense mutation Arg218Pro in the highly conserved homeodomain was identified in a 3 year old girl with semilobar HPE and global developmental delay. This *SIX3* sequence change CGG(Arg218) -> CCG(Pro) was not present in any of the tested 82 alleles of our Caucasian control population. The parents requested testing to specify the recurrence risk in a future pregnancy. This test unexpectedly revealed the clinically healthy mother to be a mosaic carrier, to our knowledge presenting the first case of maternal mosaicism for a *SIX3* mutation. The low grade mosaicism in maternal genomic DNA prepared from peripheral blood was only detected by RFLP analysis using a restriction enzyme exclusively cutting the normal allele, thus utilizing the increased sensitivity due to the formation of non cleavable *wt/mut* heterodimer ds DNA molecules. We are currently establishing a single cell assay for this particular *SIX3* mutation to evaluate the frequency of mutant cells in maternal mononuclear cells. In addition, this test could potentially be used for preimplantation genetic diagnosis to avoid the ethical issues of prenatal diagnosis associated with the prediction of the fetal phenotype and discussion of the consequences in case of an affected pregnancy.

References:

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